determined that prestin diffuses in and hops between confinement zones on the order of 1 micron in average size. We observe that depletion of membrane cholesterol increases average confinement size and decreases confinement strength. Statistical analysis of squared displacements reveals that depletion of cholesterol removes domains of intermediate size between 142 and 500 nm. From measurements of the initial increase of the mean squared deviation with time, the microscopic diffusion constant was determined to be 0.05 X 10^{-12} m^2/s, and was unchanged by cholesterol depletion. Our results suggest that membrane cholesterol affects prestin function by changing prestin crowding in confinement zones, consistent with the hypothesis that the microscale organization of prestin in the membrane influences prestin function.

1390-Pos Board B300
Nano-Scale, Microsecond Diffusion Imaging of Membrane Protein - lipid Raft Interaction in the Plasma Membrane
Yunhsiang Hsu, Arnd Pralle.
Spatial membrane domains, such as created by lipid rafts and the membrane cytoskeleton, influence membrane protein mobility and hence membrane bound processes, i.e. cell signaling. However, visualization of these lipid domains in intact cells is challenging because of their small dimension and dynamics.
We have visualized lipid raft and cytoskeleton domains by tracking the diffusion of membrane proteins with thermal noise imaging (TNI). TNI tracks the diffusion of a colloid labeled membrane protein with microsecond and nanometer precision. In addition, an optical trap confines the diffusion to a small area of the membrane providing sufficient statistics for high resolutions maps of the diffusion. We observe millisecond transient confinement zones consistent with 10nm lipid rafts which redistribute after depolymerization of the actin cytoskeleton. Also, we observed larger (~80nm) cytoskeleton caused confinement zones.

1391-Pos Board B301
Modeling the Coalescence Kinetics of Cell Surface Receptor Clusters
Kathrin Spendier, James L. Thomas, Vasudev M. Kenkre.
Cells in a multicellular organism must be ready to respond to a variety of extracellular signals such as ligands on the surface of cells or parasites. To understand these signaling mechanisms, many current experiments investigate transmembrane signaling of immunoreceptors on B cells, T cells, or mast cells, initiated by binding to specific monovalent ligands in fluid membranes. In mast cells, receptor clusters initially form at cellular protrusions through diffusion mediated trapping, undergo primarily diffusive motion with a partial directed element as well, and eventually coalesce at a finite rate to form a large central receptor patch termed synapse (Spendier et al. 2010. Biophys. J. 99:388–397). We are currently developing a coalescence theory to investigate the kinetics of receptor cluster coalescence in detail, which is important in understanding the mechanism and dynamics of cellular signaling. Our coalescence theory is split into trapping considerations, which generalize Smoluchowski's well known theory for arbitrary melding probability, and a feedback idea. Trapping considerations were developed with a unified approach constructed earlier for excitons (Kenkre and Reineker. 1982. Exciton Dynamics in Molecular Crystals and Aggregates. Springer, Berlin) to compute the particle survival probability for any dimension and motion due to a closed or open trapping surface. To build a coalescence theory, we use our trapping prescription to compute the rate at which particles disappear and solve for the time dependent trap radius through a self-consistent approach. We find that our coalescence theory for a finite melding probability is in good agreement with simulations.

1392-Pos Board B302
Time Resolved Fluorescence Anisotropy on Supported Lipid Bilayers
Christopher R. Rhodes, Jay T. Groves.
The diffusion and spatial organization of cell membrane receptor-ligand interactions, such as those involved in the formation of the T-cell immunological synapse, are important physical mechanisms of cell signaling. Although the binding kinetics within these structures have been established at the level of individual microclusters, the time-resolved interactions of single receptor-ligand pairs are generally not accessible by current optical methods. A fluorescence anisotropy method is proposed that has the potential to measure receptor-ligand kinetics without the need for intrinsic labeling of membrane receptors. By measuring the depolarization of fluorescently labeled proteins attached to supported lipid bilayers, nanosecond rotation rates can be used to characterize the proteins' microenvironment. Rotational diffusion of free and hindered model proteins were used to study the flexibility of covalent protein attachment and supported lipid bilayer properties. Progress toward single-molecule measurements of receptor-ligand kinetics in live cells, facilitated by nanoengineered substrates, will also be presented.

Membrane Receptors & Signal Transduction I

1393-Pos Board B303
Pharmacologically Distinct Ligands Induce Different States of 5-HT2AR and Trigger Different Membrane Remodeling: Implications For GPCR Oligomerization
Jufang Shan, George Khelashvili, Sayan Mondal, Harel Weinstein.
We report on microsecond-scale molecular dynamics simulations of ligand-bound serotonin 2A receptor (5-HT2AR), which demonstrate for the first time at atomic detail how ligands with different measurable effects trigger differential structural perturbations both in the GPCR and the surrounding membrane. In these simulations the full agonist (5-HT), partial agonist (LSD) and inverse agonist (Ketanserin) affect differently the GPCR structural motifs/functional microdomains (e.g., the toggle switch and the ionic lock), stabilizing different conformational states of 5-HT2AR. We find that the dynamics of 5-HT in the binding pocket, moving away from and reentering the binding pocket, are correlated with the dynamics of the ionic lock which prefers the activated state configuration when the agonist is bound. Notably, significant conformational changes occur when 5-HT is replaced by Ketanserin in the activated state, resulting in the stabilization of an inactive-like state of the receptor, consonant with the inverse agonist properties of the ligand. The different states of GPCRs induced by pharmacologically distinct ligands also induce different reorganizations of the lipid matrix surrounding the receptor, and the local membrane perturbations produce different extents of hydrophobic mismatch around the transmembrane (TM) helices of 5-HT2AR. We quantified the energetics of these perturbations with a novel computational procedure (see Sayan Mondal et al., this meeting) for quantitative modeling of anisotropic bilayer deformations around multi-helical TM insertions. To our knowledge, this is the first calculation of differences in membrane remodeling by a GPCR in complex with different ligands, establishing a link between the receptor response to different ligands and the specific membrane deformations. The mechanistic implications of these results point to modes of ligand-induced GPCR oligomerization driven by the hydrophobic mismatch between the receptor and the membrane.

1394-Pos Board B304
Molecular Dynamics Simulations of the Transmembrane Helix of the FGFR3 Receptor in POPC and DPPC
Tyler Reddy, Benjamin Hall, Alan Chetwynd, Mark S.P. Sansom.
Fibroblast growth factor receptor 3 (FGFR3) is a receptor tyrosine kinase that negatively regulates bone growth. Elevated FGFR3 activity results in achondroplasia, the most common form of human dwarfism. In the majority (~98%) of cases the underlying mutation is G380R in the FGFR3 transmembrane domain. We have used coarse-grained molecular dynamics simulations to study the dimerization behaviour of wild-type, heterodimer, and mutant homodimer 33-residue transmembrane FGFR3 constructs in both POPC and DPPC bilayers. FGFR3 dimers are stable once formed in POPC, but dissociations are observed in DPPC. All three FGFR3 constructs exhibit bimodal helix crossing angle distributions, in contrast to the strong preference for right-handed crossing in glycophorin A (GpA) control simulations. We present evidence for a primary FGFR3 dimer interface and a less stable secondary interface. The latter is more pronounced for mutant than wild-type constructs in POPC, but not in DPPC. The helix crossing angle is right-handed at the secondary dimer interface for both heterodimer and mutant homodimer FGFR3 constructs in POPC. G370, A374, and R397 are prevalent FGFR3 dimer contacts, while the same analysis procedure on GpA control simulations selects the most important interfacial residues established by experiment. We suggest subtle differences, relative to wild-type, in the dimerization properties of G380R FGFR3 transmembrane domains.

1395-Pos Board B305
Membrane Insertion and Membrane-Induced Conformational Changes of Talin F2F3 Triggering Integrin Activation
Mark J. Arcario, Emad Tajkhorshid.
Activation of the integrin αβ heterodimer plays an important role in important biological processes such as thrombus formation and tumor metastasis. A well-known activator of intergin is the cyoskeletal-associated protein talin, whose membrane association is suggested to initiate the activation process. However, atomic details of the membrane-binding event and subsequent activation of integrin are largely unknown. We have utilized a novel membrane mimetics system developed in our laboratory, in conjunction with equilibrium molecular dynamics (MD) simulations to describe the membrane-binding event of talin F2F3 and characterize its subsequent interaction with integrin β3. In addition to characterizing the membrane orientation patch of talin F2F3, we were able to elucidate a conserved, Phe-rich hydrophobic anchor which was suggested by mutational studies, but was not evident in previous structural studies.

Additionally, crystallographic studies could not explain the binding of the F3 subdomain to the membrane, which is well-characterized in mutational studies. Utilizing the highly-mobile membrane mimetics, we were able to capture large-scale conformational changes in talin F2F3 which promote the interaction of the F3 subdomain with the membrane via several residues in the β1-β2 loop. This study represents one of the first examples of membrane-induced large-scale conformational changes observed using MD simulations. Using the membrane-bound talin F2F3 subdomain, we then studied how the integrin β3 will be affected by membrane binding of talin. These simulations revealed atomistic details of the NPxY-PTB binding motif as well as formation of the membrane-proximal hydrophobic pocket. Additionally, talin F2F3 induces the bending of integrin β3, changing the angle between the cytoplasmic and transmembrane domains by approximately 50°. This buries the conserved β-Asp within the headgroup region, possibly giving a mechanism as to how the α-β salt bridge is broken and consequently how integrin is activated.

1396-Pos Board B306

Lipopolysaccharide Recognition via Toll-Like Receptor Signaling Complexes: A Large-Scale Simulation Study

Teresa Páramo, Thomas J. Piggot, **Peter J. Bond**.

Toll-like receptors (TLRs) are type I transmembrane glycoproteins that have emerged as key pathogen sensors in the mammalian innate immune response. TLR4 is of particular biomedical interest, because it mediates the response to bacterial lipopolysaccharide (LPS), a potent early indicator of microbial infection and the primary inducer of fatal septic shock syndrome. Unfortunately, subtle alterations in the structure of LPS derivatives can profoundly alter the resultant immunological response, hampering the rational design of TLR4 immunomodulators. To unravel the associated structure-activity relationships, we have performed long-timescale, all-atom molecular dynamics simulations of the signaling-active receptor complex. The system, which consists of two TLR4 molecules bound to two MD-2 lipid recognition domains, constitutes approximately half a million atoms in solvent. The conformational dynamics of the hetero-oligomer, and essential conserved loops, are highly responsive over timescales of hundreds of nanoseconds in a manner dependent upon the presence of a variety of bound "activating" or "inactivating" ligands, including e.g., hexa-acylated lipid A, the main inducer of the immunological response to LPS; its tetra-acylated precursor lipid IVa, which acts as an agonist in mice but as an antagonist in humans; and Eritoran, a strong synthetic antagonist currently undergoing clinical trials. This helps to rationalize how specificity is achieved in the activation of TLR4 signaling pathways. In addition, a novel, spontaneously "collapsed" conformation of the MD-2 fold has been identified in the lipid-free complex, consistent with previously hypothesized "clam-shell-like" gating motions in homologous mite allergen proteins. Supported by further simulations of the isolated MD-2 domain, this provides a possible mechanism for ligand gating and the prevention of TLR4 pathway stimulation in the absence of pathogenic signals.

1397-Pos Board B307

The PKa of Retinal Depends on the Conformation of the Beta-Ionone Ring

Shengshuang Zhu, Scott E. Feller.

While much attention has been placed on how methyl substituent groups, charge distribution, and protein environment influence the loss of the proton from the Schiff base in retinal during the phototransduction process, the influence of the conformation of the β-ionone ring has not been explored. Here, we examine the effect of β-ionone ring conformation, defined by the value of the C6-C7 torsion, on the retinal phototransduction process, specifically how the ring conformation influences the pKa value. We have carried out quantum chemical calculations, including a detailed examination of the effect of different basis sets and the inclusion of correlation, to predict the pKa as a function of the C6-C7 torsion angle. The results of the calculations suggest that the pKa value shifts around 2 units as the C6-C7 dihedral changes with the minimum pKa value arising from nonplanar conformations. Our high level MP2 quantum mechanical results also reveal very different torsional potential energy surfaces for the protonated and deprotonated forms of retinal, an observation with important implications for empirical force fields employed in molecular simulations of rhodopsin.

1398-Pos Board B308

Structural Dynamics of a Signalosome: The Receptor-G protein Complex

Thomas Huber, Parag Mukhopadhyay, Thomas P. Sakmar.

Heptahelical G-protein-coupled receptors (GPCRs) allosterically couple extracellular ligand binding to guanine-nucleotide exchange on intracellular G-proteins. Using the vertebrate visual system, we combined relevant existing structural data to assemble an omnibus ''signalosome'' complex model comprising ''active'' opsin, GDP-bound transducin and all post-translational modifications solvated in a membrane. In sub-microsecond molecular dynamics simulations, we observe reversible opening of the GDP-binding pocket, which is coupled to conformational changes in the receptor. In particular, transmembrane helix 6 distorts and the rotamer of conserved Trp265 toggles to a new orientation, opening a channel from the hydrocarbon core of the bilayer to the ligand-binding pocket of the receptor. Thermodynamic analysis of the retinal-binding kinetics in rhodopsin-F212A mutant demonstrates its role in the toggle switch. The simulations reveal the mechanism of allosteric coupling of agonist- and nucleotide-binding pockets in a GPCR signalosome complex, and imply supramolecular organization that reconcile existing atomic force and cryoelectron microscopy data.

1399-Pos Board B309

Metadynamics-Based Mechanistic Interpretation of Functional Crosstalk Between Serotonin 2A and Metabotropic Glutamate 2 receptors

Davide Provasi, Zheng Li, Jihyun Shim, Miguel Fribourg, José L. Moreno, Diomedes E. Logothetis, Javier Gonzalez-Maeso, Alexander D. MacKerell, Marta Filizola.

Serotonin 2A (2AR) and metabotropic glutamate 2 (mGluR2) receptors have been shown to form a functional and specific heteromeric complex in mammalian brain and in tissue culture preparations with possible implications in the psychotic symptoms of schizophrenia. Unlike non-antipsychotic drugs, clinically effective atypical antipsychotics (e.g., clozapine) that bind to 2AR increase the glutamate-mediated Gi signaling through the 2AR/mGluR2 heterodimer. The molecular mechanisms underlying these allosteric effects and functional crosstalk are unknown. Using molecular dynamics (MD) simulations enhanced with metadynamics, we investigated at the molecular level the conformational changes induced by atypical antipsychotic or non-antipsychotic 2AR ligands in atomistic representations of interacting 2AR and mGluR2 embedded in an explicit lipid-water environment. First, we sampled the conformational transitions from inactive to activated (opsin-like) models of the ligand-free transmembrane regions of 2AR or mGluR2 with adiabatic biased MD simulations. We then reconstructed the free-energy landscape of the 2AR/mGluR2 heterodimer along the predetermined transition trajectories in the presence of ligands, using a path collective variable approach based on metadynamics. The CHARMM forcefield with the CMAP backbone energy correction was used to describe the full systems. All calculations were performed using NAMD enhanced with the Plumed plug-in. Our results suggest that the conformational transitions of 2AR and mGluR2 are populated by different inactive and active metastable states of the receptors, which are differentially stabilized by antipsychotic and non-antipsychotic ligands.

1400-Pos Board B310

Investigating the Relative Stability of Opioid Receptor Homo- and Hetero-Dimers Using Biased Molecular Dynamics Methods

Jennifer M. Johnston, Davide Provasi, Marta Filizola.

The crucial role of opiates in the clinical management of pain places opioid receptors (ORs) among the most pharmacologically important members of the family A G protein-coupled receptors (GPCRs). A body of evidence, gathered over the last decade, supports the ability of all of the major subtypes of OR, the MOR, DOR and KOR, to form oligomers of homogeneous or heterogeneous composition at the plasma membrane. The discovery of unique functionality for some of these arrangements has shifted the focus of research towards the study of dimers/oligomers of ORs to aid understanding of the fundamental molecular factors governing opiate binding and selective activation of ORs. Using biased molecular dynamics (MD) methods, we aim to determine thermodynamic and kinetic details of the molecular mechanisms underlying homo- and heteromerization of ORs. We recently performed coarse-grained umbrella sampling MD simulations of DOR from Mus musculus in an explicit palmitoyl-oleoyl-phosphatidyl-choline (POPC):10% cholesterol-water environment, in order to estimate the free-energy difference between isolated and interacting protomers, from which, we were able to predict the lifetime of DOR homodimers. Our results indicated that this lifetime was of the order of a few seconds, in rough agreement with recent observations from single molecule fluorescence experiments on other rhodopsin-like GPCRs. Here, we extend this investigation to an experimentally supported, physiologically relevant heterodimer of DOR and KOR, which exhibits unique antinociceptive signaling properties that differ from those of the individual protomers. We investigated the relative stability of DOR/KOR dimers utilizing a combination of umbrella sampling and metadynamics methods, and compared the results with those obtained for homodimers. Our results suggest that the dimer stability varies depending on the protein sequence at the interface, and offer a route to testable prediction of dimerization-disrupting mutations.